SAMPLING AND REFERENCE MATERIAL REQUIREMENTS FOR SAFFRON AUTHENTICITY AND QUALITY EVALUATION EMERGED FROM THE METHODS DEVELOPED WITHIN THE COST ACTION FA1101 (SAFFRON-OMICS)

*Anastasia Kyriakoudi and Maria Z. Tsimidou**

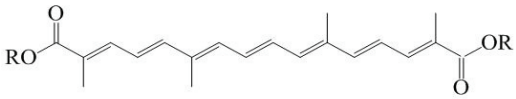
Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Food Chemistry and Technology, Thessaloniki, Greece. Emails: tsimidou@chem.auth.gr, ankyria@chem.auth.gr

Abstract – In the course of the COST Action FA1101 (Saffron-OMICS) it was evidenced how difficult can be the development of robust methods based on high throughput techniques to combat saffron adulteration and fraud without having access to samples of known history and availability of reference materials. Published data are discussed focusing on the challenges faced with regard to objectives and the characteristics of the employed techniques that addressed authenticity and quality control of the analysed samples. Importance of collaboration among different interested parties of the consortium is highlighted.

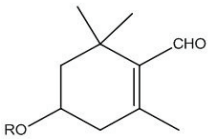
Keywords: saffron, COST Action FA1101 Saffron-OMICS, sampling, sample size, reference materials

1. INTRODUCTION

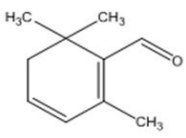
Saffron is comprised of the dried red stigmas of the plant *Crocus sativus* L. (Fig. 1), a perennial, triploid and genetically sterile plant that belongs to the *Iridaceae* family [1].



Crocetin sugar esters
R = glucose, gentiobiose, neapolitanose



Picrocrocin
R = glucose



Safranal

Fig. 1. The flower of the plant *Crocus sativus* L. (photo gallery of LFCT, AUTH, GR) and the chemical structure of the major metabolites present in the stigmas [3].

Saffron, is the highest priced agricultural product in the world with a price reaching up to 20,000 €/kg in retail market [2]. Due to its high price, saffron has been subjected to various types of fraud over the centuries. Fraudulent practices occur easier when the spice is in powder and not in whole form. Nevertheless, because consumers are not aware of "how it looks like" substitution of whole filaments with 100% other plant tissues is also frequent (Fig. 2).

Fig. 2. Common bioadulterants of saffron.

Saffron is highly valued in different cuisines for the exceptional bright-yellow hues, the distinctive bitter taste and the unique aroma that imparts to certain dishes and beverages [3]. The coloring properties are attributed to a group of water-soluble apocarotenoids rarely found in nature, the sugar esters of crocetin (8,8′-diapocarotene-8,8′-dioic acid), known as crocins. The bitter taste is mainly assigned to the colorless monoterpene glucoside picrocrocin (4-(*β*-D-glucopyranosyloxy)-

2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) whilst the aroma is attributed to many volatile compounds among which safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) prevails (Fig. 1) [4]. These sensory and appearance properties find many applications in the food and beverage industry. In a recent review, Kyriakoudi, Ordoudi, Roldán-Medina and Tsimidou [5] named the spice as a functional one because of the many biological activities (e.g. antioxidant, anti-inflammatory, anticancer) assigned to its polar extracts or individual apocarotenoids. The authors stressed that it is of great importance to highlight that «the biological actions can be claimed only if the plant material used is authentic and of high quality».

Authenticity and monitoring of food quality is of increasing concern among the food authorities because latest reports indicate unethical dangerous practices (melamine in infant formulae, horse meat with phenylbutazone in beef meat products) [6] that can affect seriously consumer health. Spices are among the top commodities that are frequently adulterated either by increasing bulk or weight (addition of foreign matter of plant, animal or inorganic origin) or by enhancing characteristic properties (e.g. addition of synthetic vanilla to lower quality product; paprika dyed with non-permitted Sudan dyes). To address these issues high throughput techniques are needed, specifically for each food category or product. The demand to combat saffron adulteration and fraud, resulted in the approval of the COST Action FA1101 Saffron-OMICS ("Omics Technologies for Crop Improvement, Traceability, Determination of Authenticity, Adulteration and Origin in Saffron). The main objectives of the Action were (i) *the analysis of the saffron genome by mapping and sequencing*, (ii) *the analysis of the saffron metabolome by the precise quantification of specific metabolites (metabolic profile) and by semi quantitative data acquired by LC-MS or ^{1}H-NMR and (bio)markers revealed by multivariate statistical tools (metabolic fingerprinting),* (iii) *the development of robust techniques for traceability, determination of authenticity and origin as well as adulteration detection, based on DNA fingerprinting and chemical fingerprinting*, (iv) *dissemination of the acquired knowledge and know-how (students, researchers, saffron growers and industry) as well as dialogue with society* [2]. To achieve these multidisciplinary goals, a considerable number of European and non-European Institutions and stake-holders (Fig. 3) worked in collaboration for the preparation, realization and implementation of achievements for more than the 4 year duration of the Action (2011-1015). The efforts continue even after its official ending mainly with dissemination activities as the present one. Research efforts also go on by various members of the network.

2. AIM OF THE STUDY

The presentation focuses on saffron sampling and reference material (RM) requirements as emerged from the methods developed within Saffron-OMICS. Difficulties encountered are highlighted and approaches to overcome them are discussed for published data taking into account objectives and characteristics of the employed techniques and those of the methods developed.

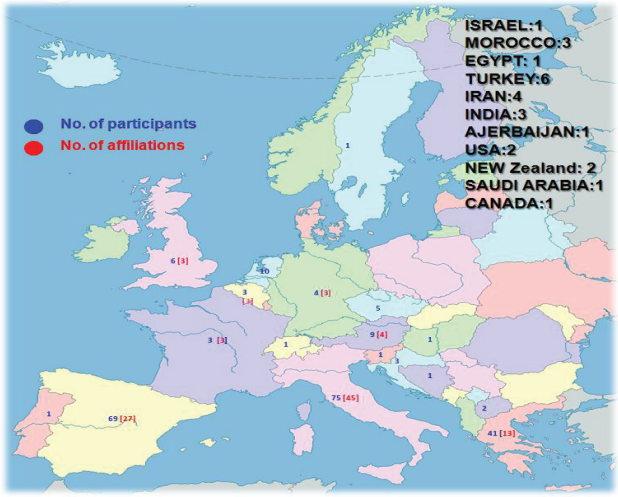


Fig. 3. The network developed in the frame of Saffron-OMICS.

3. RESULTS AND DISCUSSION

To date, saffron quality characteristics and detection of adulteration are basically examined according to ISO 3632-2 [7] analytical protocols that are in principle, univariate procedures. In the frame of the COST Action, "omic" approaches were mainly developed to detect saffron adulterants, monitor quality and traceability. Table 1 summarizes objectives, analytical approaches employed, samples and reference materials acquired, methods for data analysis introduced in the relevant publications of the consortium [8-16]. As can be seen in the data presented in Table 1, different genomic, proteomic and metabolomic approaches were elaborated. The definitions of these terms are given in Table 2 to facilitate the readers.

Table 1. Objectives, employed techniques and sampling and reference requirements of the methods developed within Saffron-OMICS.

Objectives	Employed technique*	Samples*/Sample quantity per analysis
Genomic approaches		
Determination of genetic variability of *Crocus sativus* L. and related *Crocus* species	IRAP	6 saffron samples and 30 other *Crocus* species from the WSCC (Cuenca, Spain) / not reported
Examination of the level of genetic and epigenetic variability inside the species *Crocus sativus* L.	AFLP MS-AFLP	73 Spanish saffron samples and 39 saffron samples from different EU and non-EU countries from the WSCC (Cue 200 mg per replicate
Genetic and epigenetic approaches for the detection of adulteration and auto-adulteration in saffron	AFLP MS-AFLP	2 commercial saffron samples and *Buddleja officinalis*, *Gardenia jasminoides*, *Curcuma longa*, *Carthamus tinctoriu officinalis* plant materials and *C. sativus* L. stamens and tepals as adulterants / commercial kits
Proteomic approaches		
Exploitation of proteomic tools for the complete determination of saffron proteome	^{1}D-SDS-PAGE	3 saffron samples from "Azafran de La Mancha" Association (Toledo, Spain), 1 Iranian sample from the French com (Paris, France), 1 Greek sample from Saffron Producers Cooperative of Kozani (Greece), 3 samples from Italian pr commercial sample and *Carthamus tinctorius* L. and *Gardenia jasminoides* plant materials as adulterants / 5 mg x
Metabolomic approaches		
Quality control of traded saffron	FT-IR Region (4000-400 cm^{-1})	17 saffron samples from the LFCT sample collection from Saffron Producers Cooperative of Kozani (Greece) (Collecti 2000, 2011, 2012) & 35 samples from the WSCC (Cuenca, Spain) (Collection years: 2009, 2010, 2011) (See de http://www.sciencedirect.com/science/article/pii/S0308814613016373) / 1 mg x 3 replicates
Detection of saffron quality deterioration	NMR (^{1}H-NMR spectra)	51 Greek saffron samples, 21 Spanish & 24 Iranian from the LFCT sample collection (Harvest years: 1999, 2002, 200 2008, 2009, 2010, 2011, 2012), 2 Italian samples (PDOs from L'Aquila and Sardinia) (Harvest year: 2012) (See http://www.sciencedirect.com/science/article/pii/S0963996915000368) / 4 mg per replicate
Detection plant adulterants in saffron	NMR (^{1}H-NMR spectra)	10 Greek saffron samples (organic or conventionally produced) from the Saffron Producers Cooperative of Kozani (G year: 2012) and samples of turmeric, safflower, *C. sativus* stamens & *Gardenia jasminoides* fruit extract as adulteran replicate
Traceability of commercial saffron samples	FT-IR Region (4000-400 cm^{-1}) & NMR (^{1}H-NMR spectra)	17 commercial saffron samples of unknown storage history bought in various countries (Qatar, 9; Israel, 2; KSA, FT-IR analysis: 1 mg x 3 replicates. NMR analysis: 4 mg per replicate
Detection of lower quality saffron added to fresh	PTR-MS	1 Greek saffron sample (Saffron Producers Cooperative of Kozani (Greece) and 6 saffron samples stored under u conditions for prolonged period (Production years: 2000, 2002) / 35 mg x 3 replicates

* IRAP: Inter-Retroelement Amplified Polymorfism, AFLP: Amplified Fragment Length Polymorfism, MS-AFLP: Methyl Sensitive AFLP, FT-IR: Fourier Transform-Infrared Spectroscopy, NMR: Nuclear Mag
WSCC: World Saffron and *Crocus* Collection, KSA: Kingdom of Saudi Arabia

Table 2. Definitions of the terms "genomics", "proteomics" and "metabolomics" [17].

Term	Definition
Genomics	The study of nucleotide sequences in the genome of an organism.
Proteomics	The study of the "proteome," i.e. the comprehensive analysis of a protein complement in a cell, tissue, or biological fluid at a given time.
Metabolomics	The nonbiased identification and quantification of all metabolites in a system (cell tissue, organism).

3.1. Genomic approaches

The research groups of Fernández (UCLM, Spain) and Heslop-Harrison (Lester Univ., UK) used Inter-Retroelement Amplified Polymorfism (IRAP) for the determination of genetic variability of *Crocus sativus* L. and related *Crocus* species [8]. Descriptors for *Crocus* species recently reported in literature indicate the high biodiversity in this genus [18]. The genomic approach had special demands for authentic genetic material, which were addressed thanks to the gene pool from the World Saffron and Crocus Collection germplasm bank (WSCC, Cuenca, Spain) that has been created in the frame of the AGRI GEN RES 018 Action (www.crocusbank.org) in 2007. Total genomic DNA was extracted from young leaves of single plants from *Crocus sativus* coded accessions and 30 coded accessions from 14 different *Crocus* species using standard techniques. The IRAP primers used proved to give good markers for genome assessment of diversity and relationships. Lack of variation within saffron plant was verified once more whereas analysis of genetic material from allies indicated that "interspecific hybridization occurs occasionally with consequences allowing gene flow, homogenization and hybrid speciation, leading to uncertain delimitation of species". Thus, the material of the WSCC was proved to be precious for this type of the work and it is important to consider that is available to other researchers according to a certain protocol for request.

Continuing their efforts, Fernández and collaborators from Spain and Italy used the AFLP (amplified fragment length polymorfism) and the MS-AFLP (methyl-sensitive-amplified fragment length polymorfism) techniques to examine the level of genetic and epigenetic variability inside the species *C. sativus* [9]. Green leaves, collected in a single day from 73 coded accessions originating from different Spanish areas and from 39 accessions coming from EU and non EU countries, were obtained from plants propagated and managed in the collection of WSCC. The plant material was stored at -80 °C till use. The accessions had been grown for at least 3 years under the same open field conditions. Only 200 mg of tissue powder originated from the above mentioned plant materials were needed for the DNA extraction that is fully described in the original publication. Low genetic but high epigenetic variability was observed inside the *C. sativus* species. The authors focused on the Spanish accessions and after statistical treatment of the MS-AFLP data by Factorial Correspondence Analysis (FCA), they managed to cluster, in two separate groups, these samples based on their geographical origin i.e. East and West Spain.

Moreover, Fernández and collaborators from France, Spain, Greece and Italy employed genetic and epigenetic approaches to detect cases of adulteration and auto-adulteration in saffron [10]. Commercial kits were used for the DNA extraction. The authors extracted the DNA of 2 authentic saffron samples of Italian origin obtained from producers and that of admixtures of saffron with plant material from *Buddleja officinalis*, *Gardenia jasminoides*, *Curcuma longa*, *Carthamus tinctorius* and *Calendula officinalis* at 7 different levels of addition (50%, 20%, 10%, 5%, 2%, 1% and 0.5%). Additionally, in order to address cases of auto-adulteration, the DNA of other parts of the *C. sativus* plant i.e. leaves, stamens and tepals was also extracted. Markers developed on the sequence of the plastid gene for the enzyme maturase K, were found to allow discrimination of saffron from the different plant adulterants.

3.2. Proteomic approaches

In the frame of the "Saffron-OMICS" Action, the research groups of Tsimidou (AUTH, Greece) and Mozzarelli (Parma Univ., Italy) exploited proteomic tools for the first time to characterize the proteome of saffron and to detect possible frauds [11]. This proteomic study took advantage of the material provided by producers and disseminated to the consortium partners. Proteins were extracted from

authentic saffron samples of different origin. In particular, 3 Spanish saffron samples that had been stored for 3 years after processing, 1 Iranian sample that was stored for about 3 years, 1 Greek sample stored for *ca* 3 years after processing and storage, 3 samples from Italian producers and 1 commercial sample from an Italian company were tested. Protein extraction was carried out using 5 mg of finely ground material per replicate (*n* = 3). Cases of saffron adulteration with the new generation of bioadulterants, such as *Carthamus tinctorius* L. and *Gardenia jasminoides*, were also addressed. The authors found differences in the number of detected proteins among the examined samples from different geographical origin using 1D-SDS-PAGE. Differences were also observed in the protein pattern of authentic saffron and the examined plant adulterants. A data bank for saffron proteome is under development at Parma University.

3.3. Metabolomic approaches

Quality monitoring is very important for in house control systems and in spice trade. As most of the spices, saffron is mainly consumed far away from the production area whereas different wholesalers or retailers intervene from producers to end-users. In case the product is distributed packed authentication controls and traceability measures can have a positive result. However, when saffron is sold in bulk in open markets, its quality deteriorates faster and authenticity cannot be guaranteed. Tsimidou and collaborators developed an FT-IR approach [12] that took advantage of a saffron sample collection at AUTH (Greece) of known history (harvest date, storage conditions, compositional data at different time periods). Saffron samples from WSCC were also examined. The Fourier-transform mid-infrared (FT-MIR)-data were then subjected to Principle Component Analysis, PCA) in order to monitor the quality control of saffron. FT-IR spectroscopy is a powerful technique that finds many applications in food safety, quality control and authenticity issues [19]. More specifically, in that study, the authors reported the changes that the FT-IR spectrum of saffron undergoes as a result of storage under conditions that favor decomposition of its major secondary metabolites, i.e. crocins and picrocrocin. Changes (hydrolytic or oxidative) were monitored in the range 400-4000 cm^{-1}. For this purpose a total of 52 authentic saffron samples of different origin and harvest year were examined [''reference set', *n* = 29; ''evaluation sets'', *n* = 8 and ''test set'', *n* = 15]. Reduction of data for following deterioration became feasible. The most useful for diagnostic monitoring of storage effects was found to be the band at 1028 cm^{-1}, associated with the presence of glucose moieties, along with intensities in the region 1175–1157 cm^{-1}, linked with breakage of glycosidic bonds. Only a minute amount of sample (1 mg) per replicate (*n* = 3) was required for the FT-IR analysis.

As a continuation of the above mentioned contribution and in order to overcome the limitations of FT-IR technique regarding structure elucidation, Tsimidou and Consonni (ISMAC, Italy) research groups collaborated using a ^{1}H-NMR-based metabolomic approach coupled with chemometrics [PCA and Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA)] [13]. The previous sample collection was enriched with more authentic saffron samples of different origin and harvest years, that had been stored under various conditions (*n* = 98). In this case 4 mg of ground saffron per replicate were used to prepare the samples for the NMR analysis. As stated in the publication "the Principal Component Analysis shows a clear-cut separation of samples into two groups based on the storage period regardless of the sample origin. The S-plot derived from the Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) model shows the markers for quality deterioration, i.e., sugars bound to crocetin, glucose in picrocrocin, free sugars and fatty acids. These new markers become critical for the samples that were stored for more than 4 years. The OPLS-DA model was validated with a test set and proved to be properly designed to predict the length of storage after harvest. The answer to the question set in the market of "when" and "why" a saffron sample can no longer be considered fresh is supported by our findings". Such studies are of particular importance also toward detecting cases of auto-adulteration of saffron with lower quality one and need a large sample collection of known history that can be continually updated. No references were required in the approaches described so far.

Adulteration was another issue that was addressed using metabolomic approaches in the frame of the Action by the research groups of Polisiou (AUA, Greece) and Consonni [14]. In particular, a ^{1}H-NMR approach coupled with

chemometrics (OPLS-DA, O2PLS-DA) was employed in order to detect and identify four common plant bioadulterants (i.e. turmeric, safflower, gardenia and *C. sativus* stamens) in saffron. These plant materials can be mixed with saffron to increase its weight. For the purposes of the study 10 mg from each one of the 10 authentic saffron samples, delivered by producers, who guaranteed their authenticity, were used. The method developed is reported to deal with extensive saffron frauds at a minimum level of 20% (w/w).

Traceability issues are very important in saffron trade because of the high price. Tsimidou and Consonni groups collaborated once again in a metabolic approach combining the ^{1}H-NMR and the FT-IR techniques for the traceability of commercial saffron samples [15]. This time the researchers used the established FT-IR and ^{1}H-NMR data banks from their previous works to track back the "age" of 17 commercial samples of unknown history that were labeled as "saffron". These samples had been purchased from open markets and retail shops in major saffron consuming countries by Saffron-OMICS partners and stakeholders. Chemometrics (i.e. PCA, PLS-DA, OPLS-DA) helped researchers to propose a cut-off period of 4 years beyond which a saffron sample cannot be accepted for consumption.

Another publication of the network, derived by Tsimidou and Van Ruth (Wageningen Univ., The Netherlands) research groups, focused on the volatile fraction of saffron [16]. Thus, Nenadis et al. developed a metabolomic approach using the non-destructive proton transfer mass spectrometry (PTR-MS) technique coupled with chemometrics (PCA). This approach was applied for the first time for the detection of saffron auto-adulteration. For the purposes of this work 1 authentic Greek saffron sample and 6 saffron samples stored under uncontrolled conditions for prolonged period (production years: 2000, 2002) at AUTH saffron sample bank were used. Moreover, the authors succeeded in using only a minute amount of saffron (35 mg per replicate, n = 3) for obtaining the fingerprint and they suggested that PTR-MS could be used complementary with other advanced analytical techniques to address quality and authenticity issues of saffron.

Taking into account all the above, it is of great importance to stress that for the development of those metabolomic approaches, emphasis was given on the selection of meaningful samples that derived from the sample banks of certain universities and research laboratories and the international collaboration of trusted stakeholders. Not only the kind, but also the amount of samples used in those cases (i.e. 1-200 mg) must be highlighted. These amounts are not only much lower than that required according to the protocols of ISO 3632-2 [7] to determine the physicochemical properties of saffron (>5 g), but can also provide an enormous mass of relevant information. This is very important considering the high price of the spice. Regarding the need for reference materials, this is by-passed using various chemometric tools. Such a option is of particular importance in the case of saffron because the commercial availability of reference materials is extremely limited and constitutes the reason why some laboratories isolate in-house certain metabolites to be then used as RMs [20]. The latter is a tedious, time consuming, low yield procedure that needs many tests to verify purity of the derived mixtures or individual crocins.

5. CONCLUSIONS

"Omics" techniques, such as genomics, proteomics, metabolomics, etc., constitute modern and powerful approaches towards the detection of saffron fraud and monitoring of its quality. The need for appropriate samples was stronger than that for reference materials. Relevant bodies, such as trade standards organizations, food standards agencies etc., should take into consideration and further exploit the potential of the approaches developed in the course of Saffron-OMICS COST Action in addressing serious cases of fraudulent practices.

ACKNOWLEDGEMENTS

This manuscript is part of the dissemination activities of the COST Action FA1101 (Saffron-OMICS: "Omics Technologies for Crop Improvement, Traceability, Determination of Authenticity, Adulteration and Origin in Saffron"), (2011-2015) and was prepared by the Chair of the Action Professor M. Z. Tsimidou with the assistance of the WG4 member Dr. A. Kyriakoudi. All the members of the network that worked devotedly for the publications discussed in this presentation are acknowledged.

REFERENCES

[1] J. A. Fernández, O. Santana, J.L. Guardiola, R.V. Molina, P. Heslop-Harrison, G. Borbely, et al. "The World Saffron and *Crocus* collection: strategies for establishment, management, characterization and utilization", *Genetic Resources and Crop Evolution*, vol. 58, n°. 1, pp. 125-137, January 2011.

[2] MoU of COST Action FA1101: Omics Technologies for Crop Improvement, Traceability, Determination of Authenticity, Adulteration and Origin in Saffron. European Cooperation in Science and Technology, 2011.

[3] S. Ordoudi and Tsimidou M. Saffron quality: Effect of agricultural practices, processing and storage. Dris R, Jain SM, editors. In: Production Practices and Quality Assessment of Food Crops, Volume 1. Kluwer Academic Publishers. pp. 209-260, 2004.

[4] M. Carmona, A. Zalacain and G. L. Alonso, "The chemical composition of saffron: color, taste and aroma", 1st ed., Editorial Bomarzo S.L. Albacete, ES, 2006.

[5] A. Kyriakoudi, S. A. Ordoudi, M. Roldán-Medina and M. Z. Tsimidou, "Saffron, A Functional Spice", *Austin Journal of Nutrition and Food Sciences*, vol. 3, n°, 1, pp. 1059, April 2015.

[6] M. Z. Tsimidou, S. A. Ordoudi, N. Nenadis, I. Mourtzinos. Food Fraud. In: Encyclopedia Food and Health, eds. B. Caballero, P. M. Finglas, and F. Toldrá, pp. 35-42. Oxford: Academic Press, 2016.

[7] ISO 3632-2, Saffron (*Crocus sativus* Linneaus). Part 2: Test Methods, International Organisation for Standardization, Geneva, 2010.

[8] N. F. Alsayied, J. A. Fernandez, T. Schwarzacher and J. S. Heslop-Harrison, "Diversity and relationships of *Crocus sativus* and its relatives analysed by inter-retroelement amplified polymorphism (IRAP)", *Annals of Botany*, vol. 116, pp. 359-368, September 2015.

[9] M. Busconi, l. Colli, R. A. Sanchez, M. Santaella, M. de los Mozos Pascual, O. Santana et al., "AFLP and MS-AFLP analysis of the variation within saffron Crocus (*Crocus sativus* L.) germplasm", *PLOS ONE*, pp. 1-17, April 2015.

[10] G. Soffritti, M. Busconi, R. A. Sánchez, J. M. Thiercelin, M. Polissiou, M. Roldán and J. A. Fernández, "Genetic and Epigenetic Approaches for the Possible Detection of Adulteration and Auto-Adulteration in Saffron (*Crocus sativus* L.) Spice", *Molecules*, vol. 21, pp. 343, March 2016.

[11] G. Paredi, S. Raboni, F. Marchesani, S. A. Ordoudi, M. Z. Tsimidou and A. Mozzarelli, "Insight of Saffron Proteome by Gel-Electrophoresis", *Molecules*, vol. 21, n°. 2, pp. 167, January 2016.

[12] S. A. Ordoudi, M. De los Mozos Pascual and M. Z. Tsimidou, "On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR) spectroscopy and chemometrics", *Food Chemistry*, vol. 150, pp. 414-421, May 2014.

[13] S. A. Ordoudi, L. R. Cagliani, S. Lalou, E. Naziri, M. Z. Tsimidou and R. Consonni, *"^{1}H NMR-based metabolomics of saffron reveals markers for its quality deterioration"*, Food Research International, vol. 70, pp. 1-6, April 2015.

[14] E. A. Petrakis, L. R. Cagliani, M. G. Polissiou and R. Consonni, "Evaluation of saffron (*Crocus sativus* L.) adulteration with plant adulterants by ^{1}H NMR metabolite fingerprinting", *Food Chemistry*, vol. 173, pp. 890-896, April 2015.

[15] R. Consonni, S. A. Ordoudi, L. R. Cagliani, M. Tsiangali and M. Z. Tsimidou, "On the Traceability of Commercial Saffron Samples Using ^{1}H-NMR and FT-IR Metabolomics", *Molecules*, vol. 21, n°. 3, pp 286, February 2016.

[16] N. Nenadis, S. Heenan, M. Z. Tsimidou and S. Van Ruth, "Applicability of PTR-MS in the quality control of saffron", *Food Chemistry*, vol. 196, pp. 961-967, April 2016.

[17] D. Bagchi, F. C. Lau and M. Bagchi, *Genomics, Proteomics, and Metabolomics in Nutraceuticals and Functional Foods,* Blackwell Publishing, pp. 11, pp. 201, pp. 272, 2010.

[18] R. V. Molina, J. L. Guardiola, D. García-Luis, et al., "Descriptors for *Crocus* (*Crocus* spp.)", *Bioversity International*, 74pp, ISBN-13: 978-92-9043-999-8, 2015.

[13] R. Karoui, G. Downey, C. Blecker, "Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure-quality relationships - A review", *Chemical Reviews*, vol. 110, pp. 6144–6168, October 2010.

[20] A. Kyriakoudi, A. Chrysanthou, F. Mantzouridou and M. Z. Tsimidou, **"**Revisiting extraction of bioactive apocarotenoids from *Crocus sativus* L. dry stigmas (saffron)". *Analytica Chimica Acta,* vol. 755, pp. 77-85, November 2012.